ENERGY SOURCES UTILIZED BY *VIBRIO FETUS*[1]

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Vibrio fetus is significant in the sheep and cattle industries because it causes abortion and infertility. However, little is known of the metabolism of this organism. Smith and Taylor (1919) described *V. fetus* as being relatively difficult to cultivate immediately after isolation, but after repeated transfers cultivation became less difficult. The organism produced no gas and the reaction remained neutral or slightly acid in fermented bouillon with 0.1 per cent glucose, lactose, or sucrose. A reduced oxygen tension was reported to be necessary for growth.

According to Stockman (1919), *V. fetus* can not grow either in a free supply of air or under strictly anaerobic conditions.

Plastridge and Williams (1943) found no evidence for acid production by *V. fetus* with arabinose, glucose, dextrin, raffinose, salicin, sucrose, sorbitol, or trehalose.

This organism is apparently microaerophilic (May, 1953; Reich *et al.*, 1956; Kiggins and Plastridge, 1956). *V. fetus* appears to be totally lacking in fermentative ability, and therefore is restricted to oxidative processes for obtaining energy. Since little is known of the metabolic character of microaerophilic bacteria, it was thought that a study of the compounds which could be used as energy sources would be of interest.

MATERIALS AND METHODS

Two ovine strains, 2035 and 4440, of *V. fetus* from the Montana Veterinary Research Laboratory were used in this work.

Stock cultures were maintained in tubes containing 7 ml of brain liver heart (Difco) semisolid medium which was composed of 4.6 per cent dehydrated material.

Growth studies. For determining the utilization of energy sources, a basal medium containing 0.57 per cent dehydrated brain liver heart medium, 0.5 per cent NaCl, 0.1 per cent Na_2HPO_4, and 0.05 per cent agar was used. The concentration of added energy sources was 0.2 per cent and the pH was adjusted to 7.0. Only minimal growth resulted in this basal medium. However, when a suitable energy source was added, a marked increase in growth resulted. At least 3 determinations of the growth from each compound were made.

The inoculum was grown in 20 ml of brain liver heart semisolid medium (4.6 per cent dehydrated material) contained in 150 ml pyrex milk dilution bottles which were plugged with rubber stoppers. Each experimental vessel was inoculated with 0.1 ml of 1 day old cultures of strain 2035 or 2 day old cultures of strain 4440.

The experimental cultures were grown in 25 ml of basal medium to which the various energy sources were added. These cultures were contained in 150 ml milk dilution bottles plugged with rubber stoppers and incubated at 35 C. The bottles were placed in a horizontal position during incubation. Strain 2035 was incubated for 2 days; strain 4440, for 5 days.

The amount of growth was measured with a Klett-Summerson photoelectric colorimeter using a blue filter.

Manometric studies. The ability of cellular suspensions to oxidize several compounds was determined manometrically by conventional methods (Umbreit *et al.*, 1947).

The cells were grown in a fluid medium containing 1.0 per cent tryptose, 0.5 per cent NaCl, 0.1 per cent yeast extract, and 0.1 per cent soluble starch. The cultures were incubated for 1 day at 35 C. In order to provide an adequate supply of O_2, the depth of the broth in the containers was not more than 0.7 cm. The amount of growth obtained in a liter of this culture was sufficient for 6 Warburg vessels.

The cells were centrifuged and resuspended in M/50 K_2HPO_4, pH 7.0. The cells were not washed because the oxidation of extraneous material in the suspension was not great. One ml of cell sus-

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pension was used in each vessel; the total contents were 2.3 ml. The concentrations of the cell suspensions were such that a 1:25 dilution gave a Klett reading of about 150 units. The O_2 uptake was measured in an atmosphere of air at 37 C. The CO_2 was absorbed by alkali in the center well. The final concentration of substrate in the vessels was 0.005 M (11.5 μmoles/vessel).

RESULTS

Growth studies. The amount of growth obtained with the non-nitrogenous compounds is summarized in table 1. Lactate, pyruvate, α-ketoglutarate, succinate, fumarate, and malate all served as excellent energy sources. Acetate supported good growth of strain 4440, but only slight growth of strain 2035.

Glucose, hexosediphosphate, glycerol, glycerophosphate, oxalacetate, citrate, isocitrate, oxalosuccinate, and tartrate were all unable to support appreciable growth.

The amount of growth obtained with various amino acids is shown in table 2. Aspartate, asparagine, glutamate, and proline supported growth of both strains, although a considerable difference existed in the amount of growth obtained from the two strains. Serine was utilized by strain 2035, but not by strain 4440. Glycine, alanine, threonine, phenylalanine, histidine, lysine, arginine, cysteine, methionine, leucine, and ornithine were all found to be unsuitable energy sources.

L-Serine and L-aspartate supported more growth than did an equivalent concentration of the DL-configuration of these amino acids. It is possible that the D-forms of these amino acids were not utilized.

Manometric studies. The results showing the amount of oxidation of various energy sources are summarized in table 3. Lactate, pyruvate, and fumarate were readily oxidized. The oxidation of lactate and pyruvate proceeded at an increasing rate at first. The rate then decreased and the uptake of oxygen was completed within 2 hr.

TABLE 1

Growth of Vibrio fetus with 0.2 per cent of the various non-nitrogenous compounds added to the basal medium

Compound Added	Average Turbidity (in Klett Units) of at Least 3 Determinations	
	Strain 2035, 2 days	Strain 4440, 5 days
None	36	40
Glucose	31	31
Glucose*	38	33
Hexosediphosphate*	40	38
Glycerol	38	36
Glycerophosphate	31	37
Lactate	155	116
Pyruvate	193	152
Acetate	68	144
Oxalacetate*	68	94
Citrate	31	31
Isocitrate*	46	38
Oxalosuccinate*		58
α-Ketoglutarate	183	123
Succinate	173	189
Fumarate	206	142
Malate	166	184
Tartrate	30	43

* Compound sterilized by filtration and added aseptically to the basal medium. Compounds not so marked were sterilized by autoclaving.

TABLE 2

Growth of Vibrio fetus with 0.2 per cent of various amino acids added to the basal medium

Compound Added	Average Turbidity (in Klett Units) of at Least 3 Determinations	
	Strain 2035, 2 days	Strain 4440, 5 days
None	36	40
Glycine	22	33
DL-Alanine	29	38
L-Alanine	30	45
DL-Serine	104	40
L-Serine	178	47
DL-Aspartate	69	118
L-Aspartate	119	129
Asparagine	93	191
L-Glutamate	90	227
L-Proline	101	185
Leucine	58	32
L-Ornithine	52	55
L-Arginine	36	32
Histidine	25	35
DL-Threonine	22	23
L-Lysine	43	39
DL-Phenylalanine	40	2
Cysteine*	13	30
DL-Methionine	25	3

* Some of the turbidity is probably due to the formation of cystine in the medium.

TABLE 3

Oxidative dissimilation of energy sources by strain 2035 of Vibrio fetus

Substrate (0.005 M)	O_2 Uptake, Average of at Least 2 Determinations
	μL*
Glucose	0
Hexosediphosphate	50
Glycerophosphate	−3
Lactate	262
Pyruvate	280
Isocitrate	238
Fumarate†	162

* O_2 uptake (10–30 μL) in vessels containing no added substrate has been subtracted from the total O_2 uptake.

† Experiment terminated before oxidation was complete.

The apparent oxidation of isocitrate is difficult to understand in view of the inability of this compound to support growth.

The small amount of O_2 uptake with hexosediphosphate may have been due to the oxidation of an impurity in the preparation. No attempt was made to purify this compound.

DISCUSSION

The results of this study show that only a limited number of compounds are able to serve as suitable energy sources. Seven non-nitrogenous compounds served as excellent energy sources for strain 4440; six, for strain 2035. Of the amino acids tested, only four supported good growth of strain 4440 and five supported good growth of strain 2035.

Since this organism can not grow anaerobically it might be expected to obtain its energy by oxidative processes. A correlation exists between the compounds which served as suitable energy sources and those which were oxidized by resting cell suspensions. Lactate, pyruvate, and fumarate served as excellent energy sources and were readily oxidized. Glucose, hexosediphosphate, and glycerophosphate neither supported growth nor were oxidized. The relatively few compounds that were utilized were either tricarboxylic acid cycle intermediates or compounds which could easily be introduced into this cycle. Thus the compounds which were utilized may suggest a general metabolic pathway used to obtain energy.

It is often difficult to distinguish *V. fetus* from other vibrios isolated from animals. The inability of *V. fetus* to utilize glucose would appear to be an excellent criterion for distinguishing this organism from most other vibrios.

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SUMMARY

The utilization of energy sources for the growth of *Vibrio fetus* was investigated. Lactate, pyruvate, acetate, α-ketoglutarate, succinate, fumarate, malate, aspartate, asparagine, glutamate, and proline all served as suitable energy sources under the conditions of these experiments. Glucose, hexosediphosphate, glycerol, glycerophosphate, oxalacetate, citrate, isocitrate, oxalosuccinate, tartrate, glycine, alanine, threonine, cysteine, methionine, histidine, phenylalanine, leucine, lysine, ornithine, and arginine did not support appreciable growth.

Resting cell suspensions oxidized lactate, pyruvate, isocitrate, and fumarate. Hexosediphosphate may have been oxidized slightly. Glucose and glycerophosphate were not oxidized.

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